



IN THE SPECIFICATION

At page 1, lines 4 - 9, substitute the following modified paragraph:

The present application is a continuation of U.S. Patent 6,335,201 (2002) application ~~serial no. 09/358,504~~ filed on July 21, 1999, which in turn was a continuation-in-part application of U.S. Patent 6,156,576 (2000), application ~~Serial No. 09/036,706~~, filed March 6, 1998, entitled "Fast Controllable Laser Lysis of Cells for Analysis".

At page 4, line 18 to page 5, line 8, substitute the following modified paragraph:

Further, since the concentrations of phosphorylated substrates in cells change on time scales of the order of seconds, the time resolution of the measurement, from the instant the contents of the cell are obtained to the time that the biochemical reactions are terminated, must be sub-second. Most conventional biochemical assays meet neither the temporal resolution nor the sensitivity limits required for these single cell measurements. The temporal, resolution requirement can be met through the use of apparatus described in U.S. Patent 6,156,576 (2000), application ~~serial. No. 09/036,706~~ filed March 6, 1998, and entitled, "Fast Controllable Laser Lysis of Cells for Analysis", to which this continuation-in-part application is related and which parent application is herein expressly incorporated by reference. The necessary degree of sensitivity can be achieved with traditional capillary electrophoresis (CE) methods. Lacking, until now, has been the molecular means to accurately determine the intracellular activity of one or more kinase species or other enzymes.

At page 19, line 14, add the paragraphs:

In the illustrated embodiment the contents of the cell or cellular component thereof is collected within one second or less of lysis of the cell. This is of utility when the cell is living so that the biological reactants can be analyzed in the state which they had obtained at the instant of lysis. In fact the contents are collected within 33 msec or less of lysis of the cell and it is believed that collection is delayed only by the time for the cell or cellular component to porate or open, which is believed to be a few microseconds or in the range of 1-10 microseconds of lysis of the cell.

The collection after lysis and the lysis itself is fast. The collector delivers at least a portion of the contents of the lysed cell or cellular component to the analysis device within one second of lysis of the cell or cellular component. Where applied to a living cell the contents of the lysed cell or cellular component is delivered to the analysis device within 33 msec of lysis of the cell or cellular component and in all probability within 1-10 microseconds of lysis of the cell or cellular component.

By positioning the inlet of a capillary directly above the cell prior to lysis, the cellular contents of the lysed cell are loaded into the lumen of the capillary by combination of the gravity siphon and electrophoresis at lysis. The force of the shock wave also conveys momentum to the cell fragments which drive them into the lumen of the capillary. As will be shown below the time required between the instant of the beginning of lysis and the loading within the lumen is significantly less than 33 msec.

The step of collecting at least a portion of the contents of the cell or cellular component thereof may further comprise the step of stopping the reactions of biochemical reactants disrupted from the selected cell or cellular component thereof to permit subsequent analysis of the biochemical reactants in the state which existed approximately at the time of disruption.

Where the cell or cellular component is free floating the step of controllably positioning the selected cell or cellular component in the medium in another embodiment comprises the step of temporarily holding the cell or cellular component in a position in the medium by a laser microbeam optical tweezers, or temporarily holding the cell or cellular component in a position in the medium by adhesion of a mechanical micromanipulator to the cell or cellular component.

In the illustrated embodiment the step of collecting at least a portion of the contents of the disrupted cell or cellular component thereof in the analysis device is by means of fluid flow of the medium, and in particular by means of siphon fluid flow of the medium. Collection can also be effected by means of electrophoresis through the medium, by means of force from the shock wave impacted on the contents, and by means of electroosmotic force. The use of a capillary with charged luminal walls allows the use of electroosmotic flow, and loading the cell's contents by the gravity siphon flow could be eliminated, if desired. The use of any type of mechanism beyond siphon, electrophoretic, electroosmotic and shock wave forces to collect the cell contents into a pipette is expressly included in the scope of the invention.

The substrate is collected by means of a microlumen in the capillary or microfabricated channel of a micropipette.